

Takashi MIKAWA\*: **A taxonomic study on Japanese  
sporangiferous Mucorales (5)\*\***

三川 隆\*: 日本産胞子嚢性ケカビ目菌類の分類学的研究 (5)

**Pilobolaceae** Corda, Icon. Fung. 1: 22 (1837).

Sporangiophores arising solitarily from trophocysts or substrate mycelia, usually unbranched, rarely branched. Subsporangial vesicles present or absent. Sporangia columellate, discharged or not discharged at maturity. Sporangial walls cutinized or not cutinized. Both sporangiola and monosporous sporangiola lacking. Sporangiospores smooth. Zygosporoes usually globose, formed on the surface of agar. Suspensors tong-like, coiling at the base.

Type genus: *Pilobolus* Tode ex Fries.

The Pilobolaceae dates back to Corda (1837) in which *Pilobolus* and *Pycnopodium* were included. Later in 1842, *Caulogaster* and *Chordostylum* were added to this family. However, some of them have been excluded from this family by subsequent many mycologists. Notably Hesseltine (1955) included *Pilobolus*, *Pilaira* and *Utharomyces* in this family and pointed out the diagnostic characters of this family as follows: 1) the cutinization of sporangial walls, 2) the manner of spore release, 3) zygosporoes with tong-like suspensors.

Traditionally *Phycomyces* have been placed in the Mucoraceae. As pointed out by Benjamin & Hesseltine (1959) and Hesseltine & Ellis (1973), this genus also resembles to the members of the Pilobolaceae in the presence of zygosporoes with tong-like suspensors which are initially coiled at the base and phototrophic nature of sporangiophores. In *Phycomyces blakesleeanus* and *P. nitens*, substrate mycelia swell here and there, and the swollen portion is divided by septa into several parts, each ones contain orange granular substances. This structure is distinguished from trophocyst in the following point: the trophocyst gives rise to sporangiophore, while the swollen portion does not usually give rise to sporangiophore. In the present

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paper, the swollen cell is called "trophocyst-like cell". In my opinion, the trophocyst-like cell is probably a structure homologous to the trophocyst found in the Pilobolaceae. In contrast, *Phycomyces* differs from the members of the Pilobolaceae in the absence of cutinized sporangial walls. The presence of the cutinized sporangial walls have been used as a character for separating the Pilobolaceae from the Mucoraceae. In my opinion, however, its absence cannot be used to exclude an organism from the Pilobolaceae. From these reasons, *Phycomyces* is transferred to the Pilobolaceae in the present paper.

Three genera have been hitherto known, in which only one genus, *Pilobolus* has been found in Japan. *Utharomyces* is reported as a new record from Japan and *Phycomyces* is added to this family in this paper.

### Key to genera of the Pilobolaceae

1. Sporangia hemispherical. Sporangial walls cutinized .....2
1. Sporangia globose to subglobose. Sporangial walls not cutinized .....  
.....*Phycomyces*
2. Sporangia forcibly discharged at maturity .....*Pilobolus*
2. Sporangia not shot away at maturity .....*Utharomyces*

***Phycomyces*** Kunze in Kunze & Schmidt, Mykol. 2: 113 (1823).

Sporangiophores arising solitarily from substrate mycelia, sometimes trophocyst-like cells, usually nonseptate, sometimes septate, bluish green to dark greenish olive. Subsporangial vesicles present or absent. Sporangia globose to subglobose, dark yellowish brown, not discharged at maturity. Sporangial walls smooth, not cutinized, deliquescent or dehiscent. Columellae with a collar, not constricted at the base. Zygosporoes smooth or faintly roughened. Suspensors with appendages.

Type species: *Phycomyces nitens* Kunze.

Four species have been hitherto known, in which the following two species have been found in Japan.

### Key to species

1. Sporangiospores  $13.8-32.2 \times 8.4-20.3 \mu\text{m}$  in size .....*P. nitens*
1. Sporangiospores  $8-14.5 \times 5-6 \mu\text{m}$  in size .....*P. blakesleeianus*
- 1) ***Phycomyces blakesleeianus*** Burgeff, Flora N.S. 18: 42 (1925).

Illust.: Matsushima, T. 1975. Pl. 413, fig. 1.

Hab. and Loc. coll.: from dung of *Cervi nipponis*, Nara city, Nara Pref. (Matsushima, T. 1975).

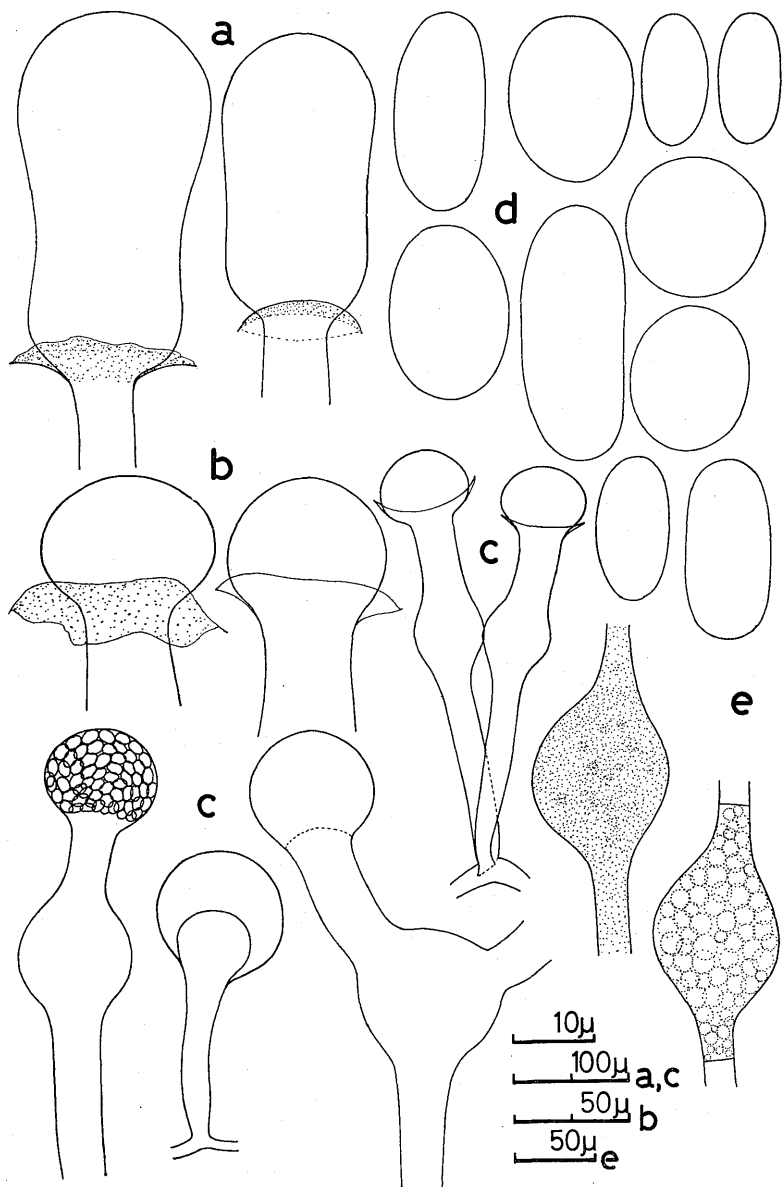
According to Benjamin & Hesseltine (1959), this species is distinguished from *P. nitens* in the following features: differences of sporangiospore size, columella shape, zygosporangium size and the optimum temperature of zygosporangium formation.

2) **Phycomyces nitens** Kunze in Kunze & Schmidt, Mykol. 2: 113 (1823).

*Ulva nitens* Agardh, Synop. Alg. Scand. 46 (1817)—*Mucor nitens* Sprengel, Syst. Veg. 4: 539 (1827)—*Phycomyces splendens* Fries, Syst. Mycol. 3: 308 (1832)—*Byssus olivacea* Winch, Trans. Nat. Hist. Soc. Northumberland. Durham & Newcastle-upon Tyne 2: 121 (1838)—*Periconia phycomyces* Bonorden Handb. Allg. Mycol. 113 (1851)—*Mucor phycomyces* Berkerley, Outl. Brit. Fung. 29, 407 (1860)—*M. ramanus* Carnoy, Bull. Soc. Roy. Bot. Belg. 9: 162 (1870)—*M. violaceus* Brefeld, Bot. Unters. Schimmelpilze 4: 56 (1881)—*Phycomyces nitens* (Agardh) Schroeter, in Cohn's Krypt.-Fl. Schlesien 3: 209 (1886)—*P. pirottianus* Morini, Malpighia 10: 89 (1896)—*P. splendens* Fries emend. Bainier, Bull. Soc. Mycol. Fr. 19: 166 (1903).

Illust.: Ito, S. 1936. fig. 114; figs. 1, a-e.

Colonies on PDA incubated at 20°C for a week dark yellowish brown, 3-4 cm high. Vegetative hyphae up to 53  $\mu$ m wide, hyaline, producing trophocyst-like cells which are intercalary, ellipsoid, ovoid or globose, with orange granular substances. Gemmae not observed. Long sporangiophore 75-168.8  $\mu$ m wide, non-septate; short sporangiophores 75-118.7  $\mu$ m wide, septate, gradually broadened upwards, sometimes forming subsporangial vesicles. Subsporangial vesicles, if present, obovoid or barrel-shaped, 97.7-187.5  $\mu$ m wide. Sporangia on long sporangiophores 100-450  $\mu$ m in diam.; sporangia on short sporangiophores 95-150  $\mu$ m in diam. Sporangial walls hyaline to light brown. Columellae on long sporangiophores pyriform, cylindric, campanulate or ovoid, 102.3-350  $\mu$ m long, 81.8-105.6  $\mu$ m wide, hyaline to light brown, sometimes containing brownish granular substances; columellae on short sporangiophores pyriform, ovoid, applanate or conical, 48-105.6  $\mu$ m long, 66.7-102.2  $\mu$ m wide, hyaline to light brown, sometimes containing dark brown to olive granular substances. Sporangiospores oblong-ellipsoid, ovoid to short ovoid, rarely globose, 13.8-32.2  $\times$  8.4-20.3  $\mu$ m, hyaline to pale yellow, sometimes with yellowish orange substances. Zygosporangia not observed.



Figs. 1. a-e. *Phycomyces nitens*. a. Columellae on long sporangiophores. b. Columellae on short sporangiophores. c. Short sporangiophores having sporangia and subsporangial vesicles. d. Sporangiospores. e. Trophocyst-like cells.

Hab. and Loc. coll.: from forest soil, Sueyoshi, Hachijo Isl. (Mikawa-no. 466); rat dung, kindly collected by Dr. Shigeru Daigobo in Suginami, Tokyo (Mikawa-no. 216); rat dung, Otsuka, Tokyo (Mikawa-no. 879); soil mixed with waste grains, Koishikawa, Otsuka, Tokyo (Inui, T. 1900 as *P. nitens* Agardh); oleaginous substrates and food stuffs, Hokkaido and Honshu (Ito, 1936); rice boiled together with red beans (=sekihan), Shibuya, Tokyo and miso-maru, Tohoku-machi, Kamikita-gun, Aomori Pref. (Kominami, 1909 as *P. nitens*).

Zygospores have been found by many mycologists including Benjamin & Hesseltine (1959) and O' Donnell *et al.* (1978). Notably O' Donnell *et al.* (1978) gave the detailed account of the zygosporogenesis in *P. nitens*.

The present isolates agreed with the description given by Benjamin & Hesseltine (1959).

During the examination of the present isolates, subsporangial vesicles were found on short sporangiophores, the occurrence is rare and no subsporangial vesicles were observed on long sporangiophores. However, the occurrence of subsporangial vesicles together with the evidence mentioned previously may lead to supposition that *Phycomyces* is regarded as a members of the Pilobolaceae.

In 1900 Inui used the name *P. nitens* Agardh for Kunze's *P. nitens*. According to Benjamin & Hesseltine (1959), this species was first described by Agardh (1817) as *Ulva nitens*. Later Kunze (1823) treated Agardh's species as a fungus and transferred *Ulva nitens* to the genus *Phycomyces*, making the new combination *P. nitens*. Therefore, Agardh is not the first person validly to publish the name *P. nitens*.

**Pilobolus** Tode ex Fries, Syst. Mycol. 2: 308 (1823).

*Pilobolus* Tode, Schrift. d. Berl. Gesell. naturf. Fr. 5: 46 (1784)—*Pycnospodium* Corda, Icon. Fung. 5: 18 (1842).

Sporangiophores arising solitarily from trophocysts, non-septate, hyaline. Sporangial vesicles always present. Sporangia hemispherical, black, forcibly discharged together with columellae. Sporangial walls smooth, cutinized. Zygospores smooth or faintly roughened. Suspensors without appendages.

Type species: *Pilobolus crystallinus* Tode ex Fries.

Seven species have been hitherto known, in which the following four species have been found in Japan.

## Key to species

1. Sporangiospores ellipsoid to oblong-ellipsoid .....2
1. Sporangiospores globose to subglobose .....3
2. Sporangiospores  $12-20 \times 6-10 \mu\text{m}$  in size .....*P. kleinii*
2. Sporangiospores  $9.7-12.8 \times 6-7.5 \mu\text{m}$  in size .....*P. crystallinus*
3. Sporangiospores with thick, two-layered walls .....*P. oedipus*
3. Sporangiospores with thin, one-layered wall .....*P. sphaerosporus*
- 1) ***Pilobolus crystallinus*** Tode ex Fries, Syst. Mycol. 2: 308 (1823).

*Pilobolus crystallinus* Tode, Schrift. d. Berl. Gesell. naturf. Fr. 5: 47 (1784)—*Mucor acicularis* Wallroth, Fl. Crypt. Germ. 2: 319 (1833).

Illust.: Indoh, H. 1962. fig. 4; Matsushima, T. 1975. Pl. 412, figs. 1-5; figs. 2, a-c.

Colonies on dung extract agar incubated at  $20^{\circ}\text{C}$  for a week hyaline, 2-3mm high. Vegetative hyphae up to  $18.4 \mu\text{m}$  wide, hyaline. Trophocysts terminal or intercalary, ellipsoid, ovoid or barrel-shaped,  $44-370 \mu\text{m}$  wide, containing orange granular substances. Gemmae not observed. Sporangioophores  $72-222 \mu\text{m}$  wide. Subsporangial vesicles ovoid, ellipsoid or barrel-shaped,  $194-547 \mu\text{m}$  wide, hyaline, with orange granular substances. Sporangia  $128-441 \mu\text{m}$  in diam. Sporangial walls dark yellowish brown, with or without reticulate ornamentation. Sporangiospores oblong-ellipsoid,  $9.7-12.8 \times 6-7.5 \mu\text{m}$ , light brown. Zygospores not observed.

Hab. and Loc. coll.: from cow dung, Sugadaira, Nagano Pref. (Mikawa-no. 947); horse dung, Nagatoro, Saitama Pref. (Mikawa-no. 900); wallaby dung, Tama Zoo, Tokyo (Mikawa-no. 118); horse dung, Tsuchiya, Fukushima Pref., horse dung, Sakado, Saitama Pref. and elephant dung, Ueno Zoo, Tokyo (Indoh, H. 1962 as *P. crystallinus* (Wiggers) Tode); dung of *Cervi nipponis*, Nara city, Nara Pref. (Matsushima, T. 1975 as *P. crystallinus* (Wiggers) Tode).

Zygospores have been found by some mycologist including Buller (1934).

The present isolates agreed with the description given by Buller (1934) and Indoh (1962) except for the size of sporangioophores and subsporangial vesicles.

This species was cited as *P. crystallinus* (Wiggers) Tode by Indoh (1962) and Matsushima (1975). On the basis of the starting-point date of nomenclature of the Mucorales, *P. crystallinus* Tode ex Fries which was validated

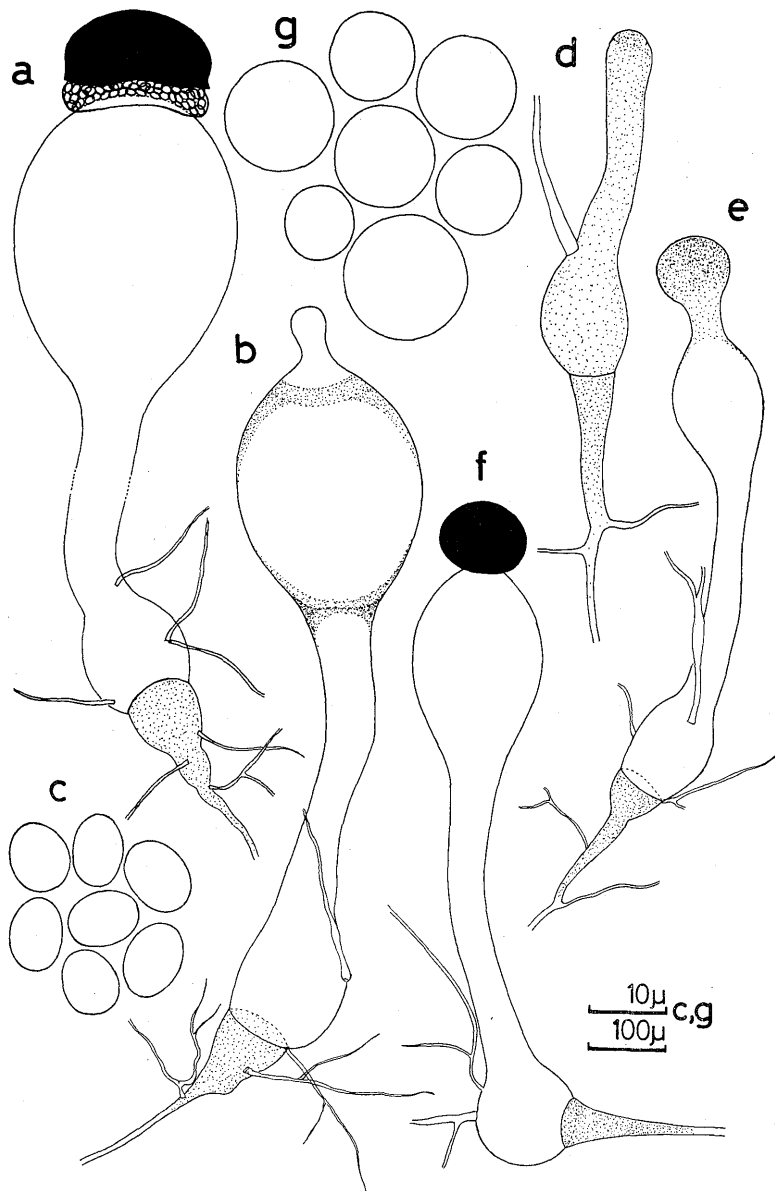


Fig. 2. a-c. *Pilobolus crystallinus*. a. Sporangiophore having a sporangium and subsporangiophore vesicle. b. A columella and subsporangiophore vesicle. c. Sporangiospores. d-g. *Pilobolus oedipus*. d, e. Early stages of sporangiophore development. f. Sporangiophore having a sporangium and subsporangiophore vesicle. g. Sporangiospores.

by Fries (1923) must be accepted.

- 2) **Pilobolus kleinii** van Tieghem, Ann. Sci. Nat. VI, 4: 337 (1876).

*Pilobolus roseus* Spegazzini, Fung. Argent. 1: 175 (1880)—*P. heterosporus* Palla, Oesterr. Bot. Zeitschr. 50: 175 (1900)—*P. pullus* Massee, Kew. Bull. No. 175-177: 160 (1901).

Hab. and Loc. coll.: from herbivorous animal dung and human excrement, Honshu and Hokkaido (Ito, S. 1936).

This species is distinguished from *P. crystallinus* by the following features: the differences of spore size and sporangiophore length.

From Japan, this species has been only reported by Ito (1936).

- 3) **Pilobolus oedipus** Montagne, Mem. Soc. Linn. d. Lyon 1-7 (1828).

*Pilobolus argentinus* Spegazzini, Fung. Argent. 1: 176 (1880).

Illust.: Indoh, H. 1962. fig. 5; figs. 2, d-g.

Colonies on dung extract agar incubated at 20°C for a week hyaline, 1mm high. Trophocysts terminal or intercalary, ovoid, globose or barrel-shaped, 81.4-141  $\mu$ m wide, containing orange granular substances. Gemmae not observed. Sporangiophores 40.6-81.2  $\mu$ m wide. Subsporangial vesicles ovoid or barrel-shaped, 115-193  $\mu$ m wide, hyaline, with orange granular substances. Sporangia 93-119  $\mu$ m in diam. Sporangial walls yellowish brown, without reticulate ornamentation. Sporangiospores globose to subglobose, 8.4-15.9  $\times$  7.8-15.3  $\mu$ m, light brown. Zygosporos not observed.

Hab. and Loc. coll.: from giraffe dung, Maruyama Zoo, Sapporo, Hokkaido (Mikawa-no. 544); cow and pig dung, Kumagaya, Saitama Pref., dog dung, Chichibu, Saitama Pref. and horse dung, Ozegahara Moor, Gunma Pref. (Indoh, H. 1962).

The present isolate agreed with the description given by Indoh (1962) except for the size of sporangiophores, subsporangial vesicles and sporangia. In my opinion, probably these differences may be due to cultural conditions.

- 4) **Pilobolus sphaerosporus** (Grove) Palla, Oesterr. Bot. Zeitschr. 50: 365 (1900).

*Pilobolus lentigerus* Corda, Icon. Fung. 1: 22 (1837)—*P. oedipus* var. *intermedia* Coemans, Bull. Acad. Belg. II. 16: 71 (1863)—*P. intermedius* (Coem.) Karsten, Mycol. Fenn. 4: 71 (1879)—*P. exiguus* Bainier, Ann. Sci. Nat. VI. 15: 81 (1883)—*P. kleinii* forma *sphaerospora* Grove, J. Bot. 22: 132 (1884)—*P. lentigerus* var. *macrosporus* Berk et de Toni, in Sacc. Syll. Fung. 7: 188



(1888)—*P. kleinii* var. *sphaerosporus* (Grove) Fischer, Rabenh. Krypt. Fl. 1: 263 (1892)—*P. borzianus* Morini, Mem. Accad. Sci. Ist. Bologna VI. 3: 386 (1906)—*P. borzianus* var. *geminatus* Morini, ibid. VI. 6: 225 (1909).

Illust.: Kominami, K. *et al.* 1952. fig. 14.

Hab. and Loc. coll.: from horse dung, Tokyo (Kominami, K. *et al.* 1952).

As pointed out by Indoh (1962), this species is similar to *P. oedipus* in many respects, but is distinguished from the latter species by spores having one-layered walls. Because such a single character has been used as a taxonomic criterion for species, it has been often confused with *P. oedipus*. More materials and examinations must be made before the limitation of both species are adequately clarified.

***Utharomyces*** Boedijn, Sydowia 12: 340 (1958).

Sporangiophores arising solitarily from trophocysts, non-septate, hyaline. Subsporangial vesicles always present. Sporangia hemispherical, black, not discharged at maturity. Sporangial walls spiny, cutinized, dehiscent. Columellae with a collar, not constricted at the base. Zygosporoes unknown.

Type species: *Utharomyces epallocaulus* Boedijn.

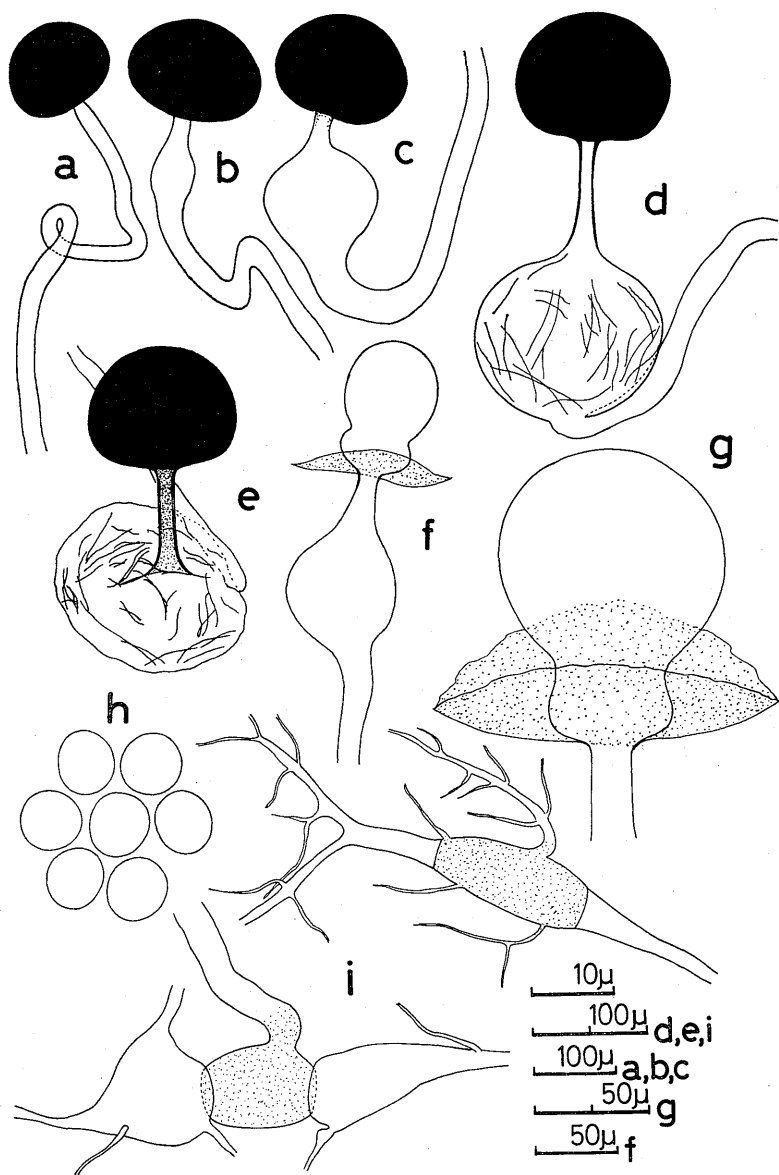
Two species have been hitherto described, in which the following one species is reported as new record from Japan in this paper.

***Utharomyces epallocaulus*** Boedijn, Sydowia 12: 342 (1958).

Illust.: figs. 3, a-i.

Colonies on LCA incubated at 20°C for 10 days dark yellowish brown, 1.5cm high. Vegetative hyphae up to 72.3  $\mu$ m wide, hyaline. Trophocysts usually intercalary, subglobose, ovoid, barrel-shaped, 81.8-104.6  $\mu$ m wide, containing orange granular substances. Gemmae not observed. Sporangiphores 10-55.4  $\mu$ m wide; the sporangiophore between sporangium and subsporangial vesicle at first hyaline and thin-walled, later becoming brownish and thick-walled in age. Subsporangial vesicles subglobose to ovoid, 104-142  $\mu$ m wide, hyaline. Sporangia 100-175  $\mu$ m in diam. Sporangial walls dark yellowish brown. Columellae usually gourd-shaped, sometimes ovoid or globose, 74.4-125  $\mu$ m long, 54.3-66.9  $\mu$ m wide, hyaline to light brown, containing brown granular substances, smooth. Sporangiosporoes globose to subglobose, 6.9-8.1  $\mu$ m in diam., hyaline to light brown. Zygosporoes not observed.

Hab. and Loc. coll.: from rat dung, Shinjuku, Tokyo (Mikawa-no. 217). New record from Japan.



Figs. 3. a-i. *Utharomyces epallocaulus*. a-c. Various stages in the formation of subsporangial vesicle. d, e. Sporangium with a collapsed subsporangial vesicle. f. A columella and subsporangial vesicle. g. Columella. h. Sporangiospores. i. Portion of vegetative hyphae showing the formation of trophocysts.

The present isolate agreed with the original description. This species is characterized by the following features: 1) it is in the habit of clinging to hyphae of other Mucorales in culture plate, 2) sporangiophores are at first erect, later become procumbent in age, 3) sporangiophores between sporangia and vesicles are at first hyaline and thin-walled, later become brownish and thick-walled in age (Figs. 3, a-e), 4) sporangia sometimes detach from sporangiophores by the collapse of subsporangial vesicles.

The second species of *Utharomyces*, *U. indicus* was described by Sarbhoy *et al.* (1974) and distinguished from *U. epallocalus* by the presence of obovoid columellae and subglobose spores. When more information of *U. indicus* is accumulated, these differences may become insufficient to separate the species.

### References

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Pilobolaceaeに所属する3属7種を報告した。これらのうち*Utharomyces epallocalus* Boedijnは新たに我国の菌類フロラに加えられた。*Phycomyces*は従来 Mucoraceaeに所属させられていたが、1) クギヌキ型接合孢子形式様式をもつこと、2) 栄養囊に似た器官をもつこと、3) まれに孢子囊下部に膨潤部を形成すること、4) 孢子囊柄が正の屈光性を示すなどの特性をもつことから、Pilobolaceaeに移すことが妥当であると結論した。